Molecular Biology Reports (2024) 51:400
https://doi.org/10.1007/s11033-024-09366-z

ORIGINAL ARTICLE

Effect of *MAOA* rs1465108 polymorphism on susceptibility to substance/alcohol use disorder: a novel PCR-RFLP assay for the detection of *MAOA* rs1465108

Dilek Kaya-Akyüzlü[1] · Selin Özkan-Kotiloğlu[2,3] · Sariye Aybüke Yıldırım[1,3] · Mustafa Danışman[4] · Mukaddes Asena Yıldırım[1,3] · İnci Özgür-İlhan[5]

Received: 15 December 2023 / Accepted: 19 February 2024 / Published online: 8 March 2024

Abstract

Background The health and social consequences of substance/alcohol use disorders are harmful. Most of the individuals cannot stop using them due to more likely their genetic background. The current study aimed both to develop a novel PCR-RFLP method for genotyping of *MAOA* rs1465108 and to analyze the effect of *MAOA* rs1465108 on the risk of alcohol (AUD), opioid (OUD) or methamphetamine (MUD) use disorders and on the depressive and anxiety symptoms in a Turkish population.

Methods and results A total of 353 individual with AUD ($n=154$), OUD ($n=160$) or MUD ($n=39$) and 109 healthy subjects were included. The intensity of anxiety and depressive symptoms and craving and opioid withdrawal were measured by appropriate scales. Logistic regression analysis revealed no association between *MAOA* rs1465108 polymorphism and substance/alcohol use disorder ($p>0.05$). Healthy subjects (3.0) had significantly lower levels of depressive symptoms than individuals with OUD (27.0), AUD (21.0) and MUD (25.5) groups. The severity of depressive symptoms was significantly higher in OUD as compared to AUD. There was a statistically significant difference between individuals with AUD, OUD and MUD in view of the average ages of first use (17, 19 and 20 years, respectively) ($p<0.05$).

Conclusions The results presented here do not support the hypothesis that *MAOA* rs1465108 is associated with substance/alcohol use disorders. The intensity of depressive symptoms could be changed according to the abused substance type. A novel PCR-RFLP was developed for genotyping of *MAOA* rs1465108 polymorphism, which could be a better option for laboratories without high technology equipment.

Keywords Opioid · Alcohol · Methamphetamine · Monoamine oxidase A · Gene polymorphism · PCR-RFLP

Introduction

Substance/alcohol use disorder is a relapsing brain disorder characterized by compulsive use of illicit drugs or alcohol in spite of harmful health and social consequences [1]. While alcohol use disorder is accepted by societies, illicit drug addiction is legally prohibited and more devastating socially. The development of addiction consists of three main stages: (i) the first use of any substance due to emotional problems or curiosity, (ii) conversion from experimentation to repeated use of a substance, and (iii) lastly substance addiction [1, 2]. In many cases, this voluntary use of a substance does not continue, whereas some individuals cannot stop the use due to various risk factors [2, 3]. Environmental and genetic factors influence the stages of the

✉ Dilek Kaya-Akyüzlü
kayadilek79@gmail.com

1 Institute of Forensic Sciences, Ankara University, Dikimevi 06590, Ankara, Turkey

2 Department of Molecular Biology and Genetics, Faculty of Science and Art, Kırşehir Ahi Evran University, Merkez/Kırşehir, Turkey

3 Graduate School of Health Sciences, Ankara University, Ankara, Turkey

4 Ankara Training and Research Hospital AMATEM Clinic, Ankara, Turkey

5 Department of Mental Health and Diseases, Faculty of Medicine, Ankara University, Ankara, Turkey

development of substance addiction at different levels. Various studies have indicated that genetic factors contribute to the transition from experimentation to addiction [4], but not to the initiation of substance use. The first use of any substance is influenced by mainly environmental factors such as the accessibility of alcohol and other illicit substances [2]. Although it is estimated that genetic contribution to substance/alcohol use disorder is approximately 40–60%, gene variants can explain only a small part of the inheritance [2, 5, 6]. Hundreds of potential genes that contribute to substance/alcohol use disorder have been identified with modest effects [7–9]. However, multiple gene variants in combination due to the genetic complexity of addiction can explain a larger proportion of genetic contribution to substance/alcohol use disorder [2, 3]. Nonetheless, the specific gene variants are largely unknown. Thus, new candidate gene variants in addiction-related genes should be identified in as diverse populations as possible to determine potential biologically causative variants across populations, which will allow to improve the treatment of addiction [2]. Variants of genes encoding metabolic enzymes and molecular targets (referred also as substance-specific genes) and those of genes playing roles in addiction neurobiology (monoamine oxidase and catechol-O-methyl transferase) have been accepted as the strongest candidate genetic contributors [1, 3, 10, 11].

The mesolimbic reward system circuits comprising the ventral tegmental area and the basal forebrain and their inputs and outputs, and neurotransmitters including norepinephrine, serotonin and dopamine are the key components of the positive reinforcing effects of the illicit substances [1, 12, 13]. The decreased functioning of monoamines such as dopamine and serotonin modulating reward, emotionality and personality traits [3] has been associated with substance use and a wide range of psychiatric disorders [14]. To date, serotonergic and dopaminergic pathway genes have been shown to be related with substance/alcohol use disorders (Bauer et al., 2015; Celorrio et al., 2016; Ruzilawati et al., 2020). However, the relationship between monoamine oxidase genes and addiction to illicit substances including opioid has been little studied [10, 15–17]. There has been no study examining the effect of the *MAOA* gene on methamphetamine use.

Monoamine oxidase (MAO) enzyme is a mitochondrial enzyme and catalyzes the oxidation of these monoamines. Thus, monoamine oxidase A could contribute to the etiology of the addiction disorders. There are two types of MAO enzyme (MAOA and MAOB) that differ from each other in their location and substrate affinity. While monoamine oxidase A (MAOA) is localized in dopaminergic neurons of the brain, MAOB is expressed most extensively in serotonergic neurons of the brain [18]. On the other hand, both of their activities have been linked with behavioral and personality preferences [19]. MAOA is encoded by the *MAOA* gene which is an X-chromosome-linked (Xp11.23 ~ 11.4) gene [3, 20]. Hitherto, there have been case–control studies that determined the risks of some *MAOA* polymorphisms such as 30 bp repeat VNTR and rs1137070 in personal traits of individuals more extensively with alcohol use disorder and to a lesser extend with opioid use disorder in a Chinese [10], in a German [21, 22], in a Finnish [23], in a Japanese [24], in an Italian [17], in a Caucasian sample [25, 26]. The focus of most of these priori studies was the 30 bp repeat VNTR polymorphism with contradictory findings. Sun et al. (2017) examined the association of *MAOA* rs1137070 with opioid use disorders and on gray matter volume [10]. However, the link between *MAOA* rs1465108 and alcohol-, opioid-, methamphetamine- use disorders remain unclear. *MAOA* rs1465108 is an intronic variant. Its functional effect has not been fully understood yet. However, Chester et al. (2015) suggested that low-functioning *MAOA* due to *MAOA* rs1465108 variation could be linked to negative urgency that is linked to a variety of maladaptive outcomes such as substance abuse [27]. Thus, the aim of the present study was to analyze the effect of *MAOA* rs1465108 on the risk of alcohol/heroin/methamphetamine use disorders as well as on the depressive and anxiety symptoms due to the association of these personality traits with *MAOA* in a Turkish population. Additionally, the techniques requiring advanced technologies and expensive consumables such as Sequenom, MassARRAY iPLEX technology and PCR followed by DNA sequencing have been used to genotype *MAOA* rs1465108 polymorphism in literature. However, a PCR-RFLP method has not been used to determine this polymorphism. Therefore, the second aim of the current study was to develop a novel PCR-PFLP method for genotyping of *MAOA* rs1465108 for research laboratories with low budget in developing countries.

Materials and methods

Case selection

This study comprised a total of 462 individuals. They were divided into 4 groups according to their substance use.

Group I (AUD) consisted of 154 Turkish volunteers who were admitted to the Alcoholism Clinic in, Department of Mental Health and Diseases of Ankara University and in AMATEM Clinic of Ankara Training and Research Hospital for detoxification treatment. International Classification of Diseases-10 (ICD-10) diagnostic criteria was used to diagnose AUD. Individuals with AUD had also fulfilled the DSM-5 criteria. Individuals with substance use disorders

(heroin, cocaine, methamphetamine etc.) were not included to this group.

Group II (OUD) comprised 160 volunteers with opioid use disorder (OUD) according to DSM–5 diagnostic criteria. They were admitted to the AMATEM Clinic for opioid maintenance treatment. Individuals with AUD or substance use disorder other than heroin were not included, which is proved by the urine drug test in AMATEM laboratory.

Group III included 39 individuals with methamphetamine use disorder (MUD) who attended to the AMATEM Clinic. They included the present study after the urine drug test showed that they only use methamphetamine, but not other illicit drugs or alcohol.

Group IV (control group) comprised healthy volunteers admitted to Blood Donation Center of İbni Sina Hospital in Ankara University. They had no diagnosis of current or past substance/alcohol addiction, and they were matched to subjects in Group I, II and III for smoking habits and gender.

In addition, all subjects in 4 groups were smokers and they were ≥ 18 years of age. Individuals with psychiatric illness (schizophrenia, psychotic disorders, severe depression, mental retardation etc.) were not included in none of the study groups. Blood samples were collected from all volunteers of 4 groups after obtaining the institutional ethics committee approval (İ03-109-22 in 2022 and I3-220-21 in 2021) and samplings were performed according to the principles of The Declaration of Helsinki. All subjects signed the written informed consent and filled a small questionnaire including questions regarding socio-demographic information.

DNA isolation and genotyping of *MAOA* rs1465108 polymorphism

Genomic DNA was isolated from 462 individuals' blood samples by Genejet Genomic DNA Purification Kit according to the manufacturer's recommendation (Thermo Fisher Scientific, USA). Isolated DNA was stored at -20 °C until PCR analysis. All isolated DNA of 462 individuals could accurately be genotyped with the novel PCR-RFLP method in the present study.

Optimization of novel PCR-RFLP assay for genotyping of *MAOA* rs1465108

In the current study, a novel PCR-RFLP method was designed to detect *MAOA* rs1465108 single nucleotide polymorphism (SNP). The designed forward and reverse primers were 5'-TGGTGACTTGCCTTCAGACT-3' and 5'-ACGA TTGCCCTATCACAGCA-3', respectively. The specificity of the novel designed primer pairs was confirmed using the Primer-BLAST program. With PCR amplification using the Techne Tc 512 PCR instrument (Thermo Fisher Scientific, USA), a 684 bp product including a A/G transition polymorphism at position 43,678,961 in the intron region (Accession No. NC_008957.2) of *MAOA* was yielded.

The PCR in a volume of 25 µl containing 10X PCR buffer (AMPLIQON, Denmark), 6.25 mM dNTP (Thermo Fisher Scientific, USA), 10 pmol each primer, 1 U Taq DNA polymerase (AMPLIQON, Denmark) and 50 ng genomic DNA was performed in a thermal cycler. In the initial denaturation step, genomic DNA was denatured at 94 °C for 5 min. The amplification cycling protocol was set at 94 °C, 60 °C, and 72 °C for 1 min each. A final extension was set at 72 °C for 10 min. PCR products (684 bp) were electrophoresed on 1% agarose gels with ethidium bromide and photographed using the Syngene Imaging System (Syngene, UK).

The RFLP (enzyme digestion) was performed in a volume of 10 µl containing 5 U *HhaI* restriction enzyme (Thermo Scientific, Lithuania), 10X Tango buffer and 5 µl PCR product. This reaction mixture was incubated at 37 °C in 1–2 h and then was visualized on 2% agarose gels with ethidium bromide and photographed using the Syngene Imaging System (Syngene, UK). Individuals with homozygous wild-type allele (A) yielded an uncut fragment (684 bp), whereas the G allele (homozygous for the variant allele) gave 2 fragments (492 bp and 192 bp). Homozygous wild type, heterozygous and homozygous variant genotypes of *MAOA* rs1465108 were verified by direct sequencing (Applied Biosystems 3730xl DNA analyzer, USA). Bioinformatic analysis was done using the SnapGene software. Schematic illustration of the novel PCR-RFLP assay for *MAOA* rs1465108 polymorphism were given in Fig. 1.

Measurement of the intensity of depression, anxiety, craving and opioid withdrawal

The individuals of all 4 groups were also asked to complete Beck Depression Inventory-II (BDI-II), Beck Anxiety Inventory (BAI), Substance Craving Scale (SCS) to assess the intensity of depression, anxiety, and craving, respectively. Opioid withdrawal was evaluated using Clinical Opioid Withdrawal Scale (COWS) in only individuals with OUD. The validity and reliability of a Turkish version of these scales was demonstrated in Turkish individuals previously [28–31].

Statistical analysis

For statistical analysis, SPSS version 20.0 was used. First, the Kolmogorov-Smirnov test was applied to detect whether the numerical data were normally distributed or not. While the mean, standard deviation, minimum and maximum values were given for normally distributed numerical data, the median and interquartile range (IQR) values were

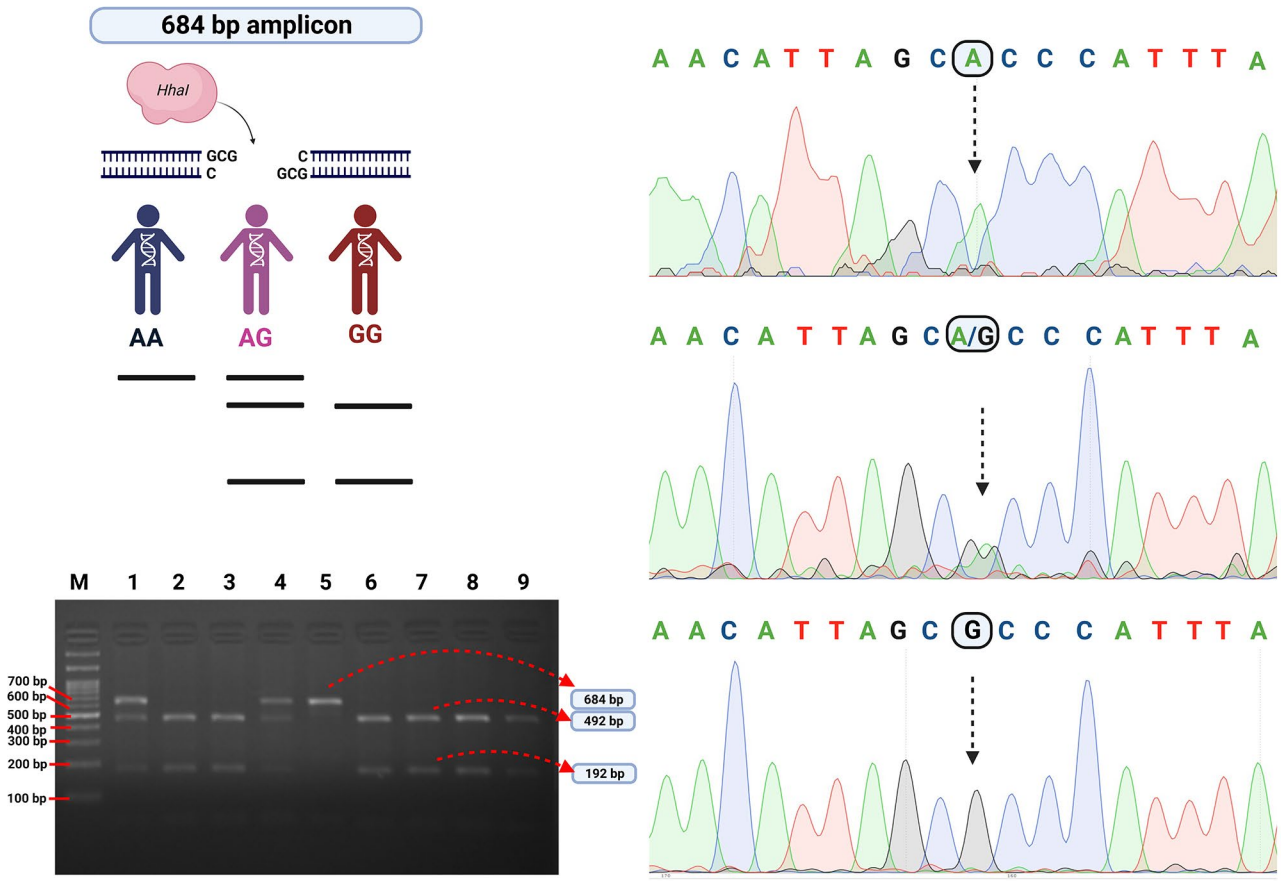

Fig. 1 Schematic illustration of the novel PCR-RFLP assay for *MAOA* rs1465108 polymorphism

given for non-normally distributed numerical data. Numbers and percentages were given for all categorical data. Genotype and allele frequencies were calculated by direct counting, and deviation from the Hardy-Weinberg Equilibrium was detected by the chi-square test for only females. Since *MAOA* gene is X-linked, all males have only one allele (A or G). Females had all 3 types (AA, AG, or GG) of *MAOA* rs1465108 genotype. Binary logistic regression analysis was used to determine the relationship between the *MAOA* rs1465108 and opioid, methamphetamine or alcohol dependence. The difference between study groups in view of socio-demographic characteristics was determined using the chi-square test. The difference between *MAOA* rs1465108 genotypes for females and for all individuals and *MAOA* rs1465108 alleles for males in view of the total scores of measurements, age onset of first substance/alcohol use, the amount of daily heroin/opioid use etc. was analyzed using Kruskal-Wallis test, Mann-Whitney U test, Student's t-test or one-way ANOVA test depending on the normal distribution of the numerical data. Post-hoc test was also used to locate the origin for any significant difference in groups. Correlation test was also used to determine whether there was a correlation between the total scores of measurements. $p < 0.05$ was considered as statistically significant.

Results

Demographics of the subjects

In total 109 controls (101 males and 8 females), 160 individuals with OUD (143 males and 7 females), 129 individuals with AUD (154 males) and 39 individuals with MUD (29 males and 10 females) were included in the current study. The median ages of the individuals with OUD, AUD, MUD and healthy subjects at the time of ascertainment were 27, 44, 29 and 34 years. A significant difference was found between these groups in view of ages ($p = 0.001$). Age onset of first substance/alcohol use (years) was 17.0 in AUD group, 19.0 in OUD group and 20.0 in MUD group and this difference between 3 groups was statistically significant ($p = 0.001$). Post hoc test showed statistically significant differences in age onset of first substance/alcohol use comparing individuals with AUD, OUD and MUD (AUD:OUD,

$p = 0.001$, AUD:MUD, $p = 0.001$). The total scores of BDI-II and BAI were also compared between individuals with AUD, OUD and MUD and controls, and a statistically significant difference was found between 4 groups ($p = 0.001$) (Fig. 2). Using post hoc test, we identified exactly which groups differ from each other for BDI-II scores (AUD/OUD/MUD:control, $p = 0.001$ and AUD:OUD, $p = 0.037$) and for BAI total scores (AUD/OUD/MUD:control, $p = 0.001$).

The median total scores of BDI-II and BAI for all groups were given in Table 1. The correlation between the total scores of measurements including BDI-II, BAI, SCS and COWS (only in OUD group) and age onset of first substance use were also analyzed (data not shown). There was a significant and positive correlation between the intensity of depression and anxiety in individuals with AUD ($p = 0.001$, $r^2 = +0.625$), with OUD ($p = 0.001$, $r^2 = +0.474$), and with MUD ($p = 0.001$, $r^2 = +0.681$). In individuals with OUD, the intensity of depression symptoms was also significantly correlated with opioid craving ($p = 0.001$, $r^2 = +0.362$). It may be noted that age onset of first opioid use was significantly and negatively correlated with the amount of daily opioid use (gr/day) in individuals with OUD ($p < 0.05$, r^2=-0.189). Table 1 also showed the frequencies of some demographic parameters such as marital, education and occupation status of individuals in all 4 groups of this study.

Frequencies of *MAOA* rs1465108 genotypes and alleles

Genotype and allele frequencies of *MAOA* rs1465108 polymorphism in individuals with AUD, OUD, MUD and in control group were shown in Table 2. These frequencies were given for total individuals as well as for females and males since there is no heterozygous genotype in males. There was not a statistically significant difference between groups in view of genotype frequencies ($p > 0.05$). In addition, there was no deviation from HWE in females of OUD, MUD and control groups.

The relationship between the *MAOA* rs1465108 genotypes and opioid, methamphetamine or alcohol dependence was examined separately for all individuals in groups, males and females by binary logistic regression analysis (Table 2). None of the *MAOA* rs1465108 genotypes or alleles were found to be associated with opioid, methamphetamine, or alcohol dependence ($p > 0.05$).

Effect of *MAOA* rs1465108 alleles on the total scores of measurements in individuals with OUD

Table 3 showed the effect of *MAOA* rs1465108 alleles on age onset of first heroin use (years), daily amount of heroin use (gr/day) as well as on the total scores of measurements (BDI-II, BAI, COWS and SCS) in all individuals with OUD ($n = 160$), in males ($n = 143$) and in females ($n = 17$). Only in females, a trend association was shown between *MAOA* rs1465108 variation and decreased age onset of first heroin use ($p = 0.06$), with a median age onset of first heroin use of 23.0 years (19.0-30.75 years) for AA+AG genotypes and 16.0 years (14.0–21.0 years) for GG genotype. This statistically significant difference was not found neither in all individuals with OUD ($p > 0.05$) nor in males with OUD ($p = 0.492$).

When the total scores of BDI-II, BAI, COWS and SCS were compared between *MAOA* rs1465108 alleles, statistically significant differences were not found in none of the subgroups (males, females, all individuals) ($p > 0.05$) (Table 3). However, it may be noted that the intensity of opioid withdrawal (5.0) and anxiety (32.0) was higher

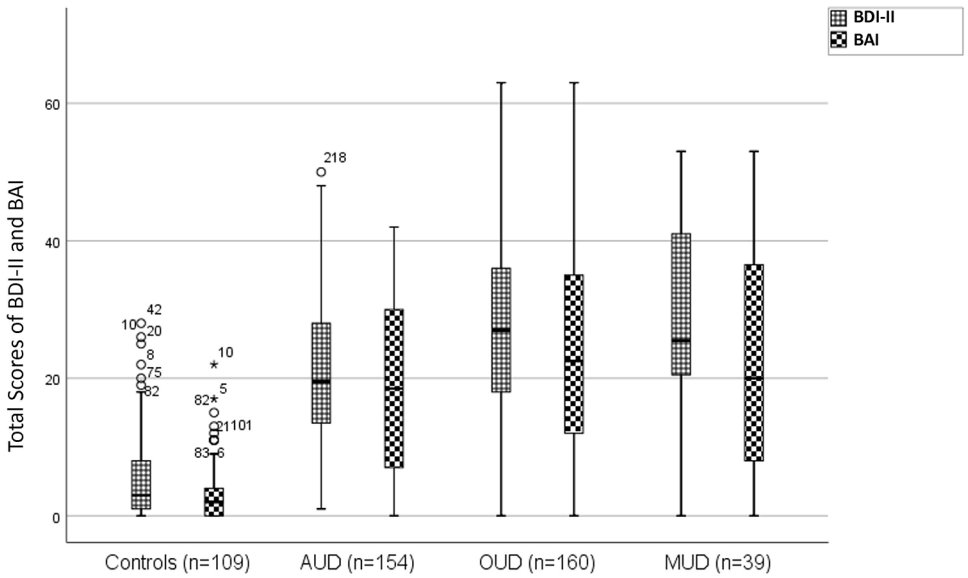

Fig. 2 Comparison of the total scores of Beck Depression Inventory-II (BDI-II) and Beck Anxiety Inventory (BAI) in healthy subjects and individuals with AUD, OUD and MUD.

Table 1 Demographic characteristics of the subjects in all groups

Characteristics	AUD ($n=154$)		OUD ($n=160$)		MUD ($n=39$)		Control ($n=109$)		*p*-value
Age (years)*	44.0 (34.75-52)		27.0 (25.0–31.0)		29.0 (24.0–34.0)		34.0 (26.5–42.0)		**0.001**
Weight (kg)*	75.0 (67.0–85.0)		68.0 (60.0-75.25)		65.0 (60.0–73.0)		82.0 (75.0–92.0)		**0.001**
Height (cm)*	175.0 (170.0-180.0)		174.0 (170.0-180.0)		171.0 (166.0-176.0)		178.0 (173.0-180)		**0.005**
Age onset (years)*	17.0 (15.0–20.0)		19.0 (17.0–23.0)		20.0 (16.0–27.0)		-		**0.001**
BDI-II*	21.0 (14.0–29.0)		27.0 (18.0–36.0)		25.5 (19.75–41.5)		3.0 (1.0–8.0)		**0.001**
BAI*	18.5 (6.5–30.0)		22.5 (12.0–35.0)		20.0 (8.0–35.0)		2.0 (0.0-4.5)		**0.001**
Marital status	n	%	n	%	n	%	n	%	**0.001**
Single	41	26.6	105	65.6	17	43.6	43	39.4	
Married	67	43.5	40	25.0	17	43.6	65	59.6	
Widow/Divorced	40	25.9	5	3.1	5	12.8	1	0.9	
NA	6	3.9	10	6.3	-	-	-	-	
Education	n	%	n	%	n	%	n	%	**0.001**
Primary	38	24.7	31	19.4	8	20.5	11	10.1	
Secondary	3	1.9	78	48.8	13	33.3	18	16.5	
High School	63	40.9	37	23.1	16	41.0	59	54.1	
Under-graduate	38	24.7	4	2.5	2	5.1	20	18.3	
Graduate	6	3.9	0	0.0	0	0.0	1	0.9	
NA	6	3.9	10	6.3	0	0.0	0	0.0	
Occupation	n	%	n	%	n	%	n	%	**0.001**
Not working	62	40.3	70	43.8	22	56.4	11	10.1	
Working	76	49.4	80	50.0	17	43.6	98	89.9	
NA	9	5.8	10	6.3	0	0.0	0	0.0	

*Given as median (IQR), N/A: Not applicable

in females with GG genotype as compared to those with AA+AG genotypes (5.0 vs. 2.5; 32.0 vs. 18.0, respectively). On the other hand, the intensity of depressive symptoms and opioid craving was lower for males with GG than those with AA+AG (Table 3).

Effect of *MAOA* rs1465108 polymorphism on the total scores of measurements in individuals with MUD

The effect of *MAOA* rs1465108 polymorphism on the total scores of measurements as well as age onset of first methamphetamine use (years), daily amount of methamphetamine use (gr/day) was also analyzed in all individuals with MUD, in females with MUD and in males with MUD (Table 4). However, no significant difference was found between *MAOA* rs1465108 genotypes ($p>0.05$). In spite of this non-significant association, it may be noted that the intensity of anxiety was higher in females having at least one A allele (32.6 ± 10.0) had higher than those with G allele (23.6 ± 21.9). On the other hand, males with A allele (14.7 ± 13.7) had lower score of BAI than those with G allele (24.1 ± 16.5). The total score of SCS was also higher in females with AA+AG (20.0 ± 6.44) as compared to those with GG (14.4 ± 9.6), but this difference was not statistically different ($p>0.05$).

Discussion

It has now been accepted that addictive disorders are genetically complex traits due to multiple sources such as genetic heterogeneity and gene–environment interactions [3]. Understanding the genetics of addiction will highlight new biologic pathways and, thus, will improve new treatment strategies of substance/alcohol use disorders as well as could decrease the numbers of addicted individuals. Although some genes have been identified through candidate gene association studies and genetic linkage mapping, there have not been yet a consensus on specific genes contributing substance use disorder. Thus, the present study was designed to contribute literature by investigating the effect of *MAOA* gene on different types of drug addiction, which has been analyzed in a limited study [10, 15–17]. Most of

Table 2 Genotype and allele frequencies of *MAOA* rs1465108 polymorphism

Groups	Genotypes			Alleles		HWE	*p*-value
	AA n (%)	AG n (%)	GG n (%)	A n (%)	G n (%)		
AUD Male ($n=154$)	**65 (42.2%)**	**-**	**89 (57.8%)**	**130 42%**	**178 58%**	-	0.564**
OUD/ overall sample *(n = 160)*	**49 (30.6%)**	**5 (3.1%**	**106 (66.3%)**	**103 32%**	**217 68%**	-	0.663*
Male ($n=143$)	46 (32.4%)	-	96 (67.6%)	92 32%	192 68%	-	0.844**
Female ($n=17$)	3 (16.7%)	5 (27.8%)	9 (55.6%)	11 31%	25 69%	$\chi2=2.15$ $p=0.14$	0.554***
MUD/ overall sample ($n=39$)	**12 (30.8%)**	**3 (7.7%)**	**24 (61.5%)**	**27 35%**	**51 65%**	-	0.745*
Male ($n=29$)	10 (34.5%)	-	19 (65.5%)	20 34%	38 66%	-	0.989**
Female ($n=10$)	2 (20%)	3 (30%)	5 (50%)	**7 35%**	13 65%	$\chi2=1.16$ $p=0.28$	0.687***
Control group/ overall sample ($n=109$)	**38 (34.9%)**	**4 (3.7%)**	**67 (61.5)**	**80 37%**	**138 63%**	-	-
Male ($n=101$)	37 (36.6%)	-	64 (63.4%)	74 37%	128 63%	-	-
Female ($n=8$)	1 (12.5%)	4 (50.0%)	3 (37.5%)	6 37%	10 63%	$\chi2=0.03$ $p=0.85$	-

*All individuals with OUD and MUD were compared with overall control group

**Only male individuals with AUD, OUD or MUD were compared with only males of control group

*** Only female individuals with OUD or MUD were compared with only females of control group

these previous studies have focused on *MAOA* variable-number of tandem repeat (VNTR) polymorphism in AUD [23, 32–35]. As for studies regarding substance use disorder, Fite et al. (2019) conducted a study with students and they indicated that *MAOA* VNTR variants influenced the polysubstance use with the effect of childhood emotional or physical abuse in a sex-specific fashion [16]. Sun et al. (2017) observed that *MAOA* rs1137070 C allele is associated with heroin addiction in a study with Chinese heroin abusers ($n=1035$) and healthy controls ($n=2553$) [10]. Chien et al. (2010) also analyzed the effect of *MAOA* gene on the susceptibility of heroin addiction in Chinese men, but with *MAOA* promoter VNTR polymorphism [15]. In contrast to Sun's study, an association between heroin addiction and *MAOA* variation was not found in Chien's study. In consistent with Chien et al. (2010), Gerra et al. (2004) could not find a difference between Caucasian heroin dependent males ($n=104$) and healthy volunteers ($n=95$) in view of the frequencies of *MAOA* VNTR repeat alleles [17]. However, they reported that 3-repeat allele (low activity) of the *MAOA* VNTR could contribute to variation in susceptibility to aggressiveness and aggressive-criminal behavior. There has been no study regarding the relationship between MUD and *MAOA*. According to the few numbers of previous studies, it is not clear whether *MAOA* variation has an effect on the vulnerability to substance use disorder. Thus, it seems that there is a need for new studies with different *MAOA* polymorphisms in different populations. To the best of our knowledge, there has been no study examining the association of *MAOA* rs1465108 polymorphism with opioid, methamphetamine, and alcohol use disorders in Caucasians.

MAOA rs1465108 polymorphism is an intronic variant (intron 1). Its functional role in gene expression has not been established yet. Chester et al. (2015) reported that A allele is associated with greater aggression due to the increased negative urgency that is one facet of impulsivity [27]. They hypothesized that the A allele could be the low-functioning allele since increased aggression has been linked to low functioning the of *MAOA* gene in both animals [36] and human studies [37, 38]. Other studies with *MAOA* rs1465108 polymorphism reported no association with autism spectrum disorder [39] and obsessive-compulsive disorder [40]. On the other hand, Karmakar et al. (2014) found that the *MAOA* rs1465108 G allele contributes to the etiology of attention deficit hyperactivity disorder (ADHD) and ADHD-associated conduct disorder in the Han-Chinese population. They also reported that the *MAOA* rs1465108 G allele did not change the function of the enzyme *in silico* analysis [41]. However, Karmakar et al. (2017) could not find this effect of *MAOA* rs1465108 on ADHD-associated behavioral traits including hyperactivity and inattention in Indo-Caucasoid boys [42]. In the current study, we could

Table 3 Comparison of individuals with OUD according to their *MAOA* rs1465108 genotypes in view of some parameters related to heroin use

Parameters	MAOA rs1465108							
	Total ($n=160$)				Males ($n=143$)		Females ($n=17$)	
	GG ($n=106$)	AA+AG ($n=54$)	GG+AG ($n=111$)	AA ($n=49$)	A allele ($n=42$)	G allele ($n=91$)	AA+AG ($n=8$)	GG ($n=9$)
Age onset (years)	19.0 (17.0–23.0)	19.5 (17.0-23.25)	19.0 (17.0–23.0)	19.0 (17.0-22.5)	19.0 (16.8–22.0)	20.0 (17.0–23.0)	23.00 (19.00-30.75)	16.00 (14.00–21.00)
p-value	$p=0.854$ U=2454.0 Z=-0.184		$p=0.847$ U=2315.5 Z=-0.193		$p=0.492$ U=1769.5 Z=-0.687		$p=0.06$ U=16.5 Z=-1.880	
Heroin use (mg/day)	3.0 (1.5-5.0)	2.0 (1.0–5.0)	3.0 (1.5-5.0)	2.0 (1.0-4.5)	2.00 (1.0-4.25)	3.0 (1.375–4.25)	2.5 (1.625-5.0)	3.0 (1.5-5.0)
p-value	$p=0.501$ U=2310.5 Z=-0.672		$p=0.434$ U=2154.0 Z=-0.782		$p=0.525$ U=1762.0 Z=-0.636		$p=0.586$ U=30.5 Z=-0.544	
COWS	3.0 (0.0–6.0)	2.5 (0.0–6.0)	3.0 (0.0–6.0)	2.0 (0.0–6.0)	2.5 (0.0–6.0)	3.0 (0.0-5.25)	2.5 (0.25–6.25)	5.0 (1.5-9.0)
p-value	$p=0.783$ U=2407.5 Z=-0.275		$p=0.505$ U=2181.0 Z=-0.667		$p=0.968$ U=1882.0 Z=-0.040		$p=0.384$ U=27.0 Z=-0.870	
BAI	22.0 (13.0–35.0)	23.0 (12.0–36.0)	22.0 (13.25–34.75)	23.5 (12.0-36.5)	24.0 (10.0–36.0)	21.5 (12.0-34.25)	18 (14.25–47.5)	32.0 (20.0–38.0)
p-value	$p=0.663$ U=2318.5 Z=-0.436		$p=0.658$ U=2182.0 Z=-0.443		$p=0.802$ U=1794.5 Z=-0.251		$p=0.289$ U=25.0 Z=-1.060	
BDI-II	27.8 ± 12.6 (0.0–58.0)	26.5 ± 13.9 (0.0–63.0)	28.4 ± 12.9 (0.0–63.0)	25.0 ± 12.9 (0.0–60.0)	25.2 ± 13.3 (0.0–60.0)	27.9 ± 12.7 (0.0–58.0)	33.75 ± 15.64 (19.0–63.0)	26.44 ± 11.8 (9.0–47.0)
p-value	t=-0.561 $p=0.576$		t=-1.464 $p=0.145$		t=-1.153 $p=0.251$		t=1.095 $p=0.291$	
SCS	17.0 ± 8.0 (0.0–32.0)	18.3 ± 8.4 (0.0–32.0)	17.4 ± 8.0 (0.0–32.0)	17.7 ± 8.6 (0.0–32.0)	17.5 ± 8.8 (0.0–32.0)	16.9 ± 8.1 (0.0–32.0)	22.6 ± 4.4 (17.0–29.0)	17.22 ± 8.21 (0.0–24.0)
p-value	t=0.943 $p=0.347$		t=0.206 $p=0.837$		t=0.341 $p=0.734$		t=1.657 $p=0.118$	

Table 4 Comparison of individuals with MUD according to their *MAOA* rs1465108 alleles in view of some parameters

Parameters	MAOA rs1465108					
	Total ($n=39$)		Male ($n=29$)		Female ($n=10$)	
	AA+AG ($n=15$)	GG ($n=24$)	AA ($n=10$)	GG ($n=19$)	AA+AG ($n=5$)	GG ($n=5$)
Age onset of first heroin use (years)	19.1 ± 6.5 (12.0–34.0)	23.25 ± 8.03 (13.0–45.0)	19.5 ± 7.65 (12.0–34.0)	24.0 ± 8.7 (13.0–45.0)	18.2 ± 3.7 (13.0–23.0)	20.4 ± 4.1 (17.0–27.0)
p-value	t=-1.699 $p=0.098$		t=-1.376 $p=0.180$		t=-0.891 $p=0.399$	
Amount of meth/per day (mg)	1.0 (1.0–4.0)	1.5 (1.0–3.0)	1.0 (0.9–4.25)	1.0 (1.0–2.0)	2.0 (1.0–6.0)	2.75 (0.75–4.75)
p-value	$p=0.818$ U=165.0 Z=-0.230		$p=0.981$ U=94.5 Z=-0.024		$p=1.00$ U=10.0 Z=0.00	
BAI	20.7 ± 15.0 (0.0–48.0)	24.0 ± 17.3 (0.0–53.0)	14.7 ± 13.7 (0.0–43.0)	24.1 ± 16.5 (0.0–53.0)	32.6 ± 10.0 (20.0–48.0)	23.6 ± 21.9 (4.0–50.0)
p-value	t=-0.616 $p=0.542$		t=-1.538 $p=0.136$		t=0.836 $p=0.427$	
BDI-II	12.1 ± 14.1 (0.0–46.0)	30.4 ± 13.5 (8.0–53.0)	22.9 ± 13.5 (0.0–46.0)	30.0 ± 13.6 (10.0–53.0)	32.0 ± 14.8 (11.0–45.0)	31.6 ± 14.7 (8.0–47.0)
p-value	t=-0.899 $p=0.375$		t=-1.274 $p=0.215$		t=0.043 $p=0.967$	

not show the effect of *MAOA* rs1465108 variation on opioid, methamphetamine and alcohol use disorders in a Turkish population. These discrepancies among limited previous studies including ours indicated that more research is necessary to determine the functional importance of the *MAOA* rs1465108 on addictive disorders in human studies.

Since both MAOA and addictive disorders have been linked to personality traits, we also examined whether MAOA could contribute to substance/alcohol use by affecting the intensity of depressive and anxiety symptoms. However, the results presented here do not support this hypothesis in any of the groups using opioid, methamphetamine or alcohol. There were also no significant differences between *MAOA* rs1465108 genotypes in view of the total scores BDI-II and BAI in the OUD group. However, it may be noted that the intensity of opioid withdrawal (5.0) and anxiety (32.0) was higher in females with GG genotype as compared to those with AA+AG genotypes (5.0 vs. 2.5; 32.0 vs. 18.0, respectively) in OUD group, but this differences between *MAOA* rs1465108 genotypes were not statistically significant due to the small number of female individuals in the current study. We believe that our findings regarding the effect of *MAOA* rs1465108 on withdrawal and anxiety in females with OUD warrant further investigation on a large female group. If this effect could be proven, it can be speculated that the G (variant) allele of the *MAOA* rs1465108 may be the low-functioning allele in accordance with findings of Karmakar et al. (2014), but contrary to suggestion of Chester et al. (2015). On the other hand, the intensity of depressive symptoms and opioid craving was lower in males with GG than those with AA+AG. The different effects of *MAOA* rs1465108 genotypes on craving withdrawal, anxiety and depression demonstrated that MAOA could function on addictive disorder-related traits in a gender-specific fashion.

Consistent with Currie et al. (2005) [43], in the current study, the intensity of depressive symptoms was higher in individuals with either alcohol or substance (opioid or methamphetamine) use disorders as compared to healthy subjects. The comorbidity between depression and AUD and/or SUD is highly prevalent [43–46]. This comorbidity can cause high treatment failures [44, 47]. Our finding demonstrating the positive and significant correlation between the intensity of depressive symptoms and opioid craving in OUD group supported previous studies. Furthermore, Jordans et al. (2019) reported that psychotherapy improved the treatment success in patients with both AUD and depression [48]. In addition, in the current study, we showed that the severity of depressive symptoms was higher in individuals OUD as compared to those with AUD, which is consistent with Anand et al. (2019) reporting a significant correlation with the intensity of depressive symptoms and the abused substance type due to their neurobiological effects [49]. The underlying mechanisms of this comorbidity has not been still understood, but most likely due to the overlapping neurobiological and genetic factors [50]. In one of our previous studies, we demonstrated that *PDYN* gene variations (rs2281285 and rs2225749) contributes to increase the intensity of depressive symptoms. On the other hand, in the current study, we could not show the effect of *MAOA* rs1465108 polymorphism on depressive symptoms in individuals with AUD, OUD or MUD.

The average ages of first alcohol, opioid and methamphetamine use were 17, 19 and 20 years, respectively, in the current study and this difference between groups was statistically significant after post-hoc test. Alcohol use is not illegal, and it is easier to get it for teenagers as compared to illicit drugs. Thus, the age of first alcohol use could be lower than the ages of methamphetamine or opioid use. Since methamphetamine use is rapidly increasing in Turkey as compared to opioid in recent years, the age of first methamphetamine use could be likely higher than the age of first opioid use. In addition, there was not a statistically significant difference between *MAOA* rs1465108 genotypes in view of the age of first alcohol/substance use. All in all, we suggested that the difference between the average ages of first alcohol, opioid and methamphetamine use could be due to sociocultural factors.

MAOA rs1465108 polymorphism was detected using different techniques such as Sequenom MassARRAY iPLEX technology [14, 27, 40], PCR followed by DNA sequencing [39, 41, 42] so far. These techniques require expensive consumables and reagents as well as advanced technologies. When compared to these techniques, PCR-RFLP is still a fundamental, sensitive, rapid and inexpensive method to detect single nucleotide polymorphisms in research laboratories in developing countries. Thus, we developed a novel PCR-RFLP method to genotype *MAOA* rs1465108 variation indefectibly. The reliability of this novel method was verified by direct sequencing. Then, it was successfully performed to blood samples of 462 whole blood samples of volunteers. Thus, the developed PCR-RFLP method could be a good option for laboratories with low budgets.

The major limitation of the current study is the small number of females with OUD, AUD and MUD. The frequency of substance/alcohol use was low in Turkish females as compared to males due to economic and social factors. We believe that our findings regarding inter-individual variability in the intensity of anxiety and opioid withdrawal warrant further investigation in studies with more females with substance use disorder from different populations.

In conclusion, the current study investigated the effect of *MAOA* rs1465108 polymorphism on opioid, methamphetamine and alcohol use disorders as well as on the intensity

of depressive and anxiety symptoms. No relationship was found between this polymorphism and substance/alcohol use disorder in a Turkish population. In females with OUD, a trend association was detected between the age onset of first opioid use and *MAOA* rs1465108 polymorphism. However, further studies are needed to find out the exact role of *MAOA* rs1465108. In addition, a novel and practical PCR-RFLP assay was developed for the determination of *MAOA* rs1465108 polymorphism, which could be a better option for research laboratories without high technology equipment.

Author contributions DKA developed the study concept, performed the statistical analysis and participated in the article preparation. SÖK performed genetic analysis and contributed to the writing of manuscript. SAY and MAY performed genetic analysis. MD collected venous blood samples and demographic data of all subjects. İÖİ contributed to the concept of the study.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval Each subject who were eligible for the study provided written informed consent and approval (İ03-109-22 in 2022 and I3-220-21 in 2021) for the use of human subjects was obtained from the institutional ethics committee.

Conflict of interest The authors declared no conflict of interest.

References

1. Barata PC, Oliveira CFP, Lima de Castro S, Rocha da Mota AMP (2019) A systematic review on Substance Addiction: medical diagnosis or morality flaw? Eur J Psychiatry 33(4):143–151. https://doi.org/10.1016/j.ejpsy.2019.07.001
2. Bierut LJ (2011) Genetic vulnerability and susceptibility to substance dependence. Neuron 69(4):618–627. https://doi.org/10.1016/j.neuron.2011.02.015
3. Ducci F, Goldman D (2012) The genetic basis of addictive disorders. Psychiatr Clin North Am 35(2):495–519. https://doi.org/10.1016/j.psc.2012.03.010
4. Vink JM, Willemsen G, Boomsma DI (2005) Heritability of smoking initiation and nicotine dependence. Behav Genet 35(4):397–406. https://doi.org/10.1007/s10519-004-1327-8
5. Kendler KS, Karkowski LM, Neale MC, Prescott CA (2000) Illicit psychoactive substance use, heavy use, abuse, and dependence in a US population-based sample of male twins. Arch Gen Psychiatry 57(3):261–269. https://doi.org/10.1001/archpsyc.57.3.261
6. Koob GF, Volkow ND (2016) Neurobiology of addiction: a neurocircuitry analysis. The lancet. Psychiatry 3(8):760–773. https://doi.org/10.1016/S2215-0366(16)00104-8
7. Al-Eitan LN, Rababa'h DM, Alghamdi MA (2021) Genetic susceptibility of opioid receptor genes polymorphism to drug addiction: a candidate-gene association study. BMC Psychiatry 21(1):5. https://doi.org/10.1186/s12888-020-03006-z
8. He L, Deng T, Luo HS (2015) Genetic polymorphism in alcohol dehydrogenase 2 (ADH2) gene and alcoholic liver cirrhosis risk. Int J Clin Exp Med 8(5):7786–7793
9. Vereczkei A, Barta C, Magi A, Farkas J, Eisinger A, Király O, Belik A, Griffiths MD, Szekely A, Sasvári-Székely M, Urbán R, Potenza MN, Badgaiyan RD, Blum K, Demetrovics Z, Kotyuk E (2022) FOXN3 and GDNF polymorphisms as common genetic factors of Substance Use and addictive behaviors. J Personalized Med 12(5):690. https://doi.org/10.3390/jpm12050690
10. Sun Y, Liu L, Feng J, Yue W, Lu L, Fan Y, Shi J (2017) MAOA rs1137070 and heroin addiction interactively alter gray matter volume of the salience network. Sci Rep 7:45321. https://doi.org/10.1038/srep45321
11. Tikkanen R, Sjöberg RL, Ducci F, Goldman D, Holi M, Tiihonen J, Virkkunen M (2009) Effects of MAOA-genotype, alcohol consumption, and aging on violent behavior. Alcohol Clin Exp Res 33(3):428–434. https://doi.org/10.1111/j.1530-0277.2008.00853.x
12. Popescu A, Marian M, Drăgoi AM, Costea RV (2021) Understanding the genetics and neurobiological pathways behind addiction (review). Experimental Therapeutic Med 21(5):544. https://doi.org/10.3892/etm.2021.9976
13. Winger G, Woods JH, Galuska CM, Wade-Galuska T (2005) Behavioral perspectives on the neuroscience of drug addiction. J Exp Anal Behav 84(3):667–681. https://doi.org/10.1901/jeab.2005.101-04
14. Groenman AP, Greven CU, van Donkelaar MM, Schellekens A, van Hulzen KJ, Rommelse N, Hartman CA, Hoekstra PJ, Luman M, Franke B, Faraone SV, Oosterlaan J, Buitelaar JK (2016) Dopamine and serotonin genetic risk scores predicting substance and nicotine use in attention deficit/hyperactivity disorder. Addict Biol 21(4):915–923. https://doi.org/10.1111/adb.12230
15. Chien CC, Lin CH, Chang YY, Lung FW (2010) Association of VNTR polymorphisms in the MAOA promoter and DRD4 exon 3 with heroin dependence in male Chinese addicts. World J Biol Psychiatry: Official J World Federation Soc Biol Psychiatry 11(2 Pt 2):409–416. https://doi.org/10.3109/15622970903304459
16. Fite PJ, Brown S, Hossain W, Manzardo A, Butler MG, Bortolato M (2019) Tobacco and cannabis use in college students are predicted by sex-dimorphic interactions between MAOA genotype and child abuse. CNS Neurosci Ther 25(1):101–111. https://doi.org/10.1111/cns.13002
17. Gerra G, Garofano L, Bosari S, Pellegrini C, Zaimovic A, Moi G, Bussandri M, Moi A, Brambilla F, Mameli A, Pizzamiglio M, Donnini C (2004) Analysis of monoamine oxidase A (MAO-A) promoter polymorphism in male heroin-dependent subjects: behavioural and personality correlates. J Neural Transmission (Vienna Austria: 1996) 111(5):611–621. https://doi.org/10.1007/s00702-004-0129-8
18. Westlund KN, Denney RM, Rose RM, Abell CW (1988) Localization of distinct monoamine oxidase A and monoamine oxidase B cell populations in human brainstem. Neuroscience 25(2):439–456. https://doi.org/10.1016/0306-4522(88)90250-3
19. Harro J, Oreland L (2016) The role of MAO in personality and drug use. Prog Neuro-psychopharmacol Biol Psychiatry 69:101–111. https://doi.org/10.1016/j.pnpbp.2016.02.013
20. Sabol SZ, Hu S, Hamer D (1998) A functional polymorphism in the monoamine oxidase A gene promoter. Hum Genet 103(3):273–279. https://doi.org/10.1007/s004390050816
21. Samochowiec J, Lesch KP, Rottmann M, Smolka M, Syagailo YV, Okladnova O, Rommelspacher H, Winterer G, Schmidt LG, Sander T (1999) Association of a regulatory polymorphism in the promoter region of the monoamine oxidase A gene with antisocial

alcoholism. Psychiatry Res 86(1):67–72. https://doi.org/10.1016/s0165-1781(99)00020-7
22. Schmidt LG, Sander T, Kuhn S, Smolka M, Rommelspacher H, Samochowiec J, Lesch KP (2000) Different allele distribution of a regulatory MAOA gene promoter polymorphism in antisocial and anxious-depressive alcoholics. J Neural Transmission (Vienna Austria:1996 107(6):681–689. https://doi.org/10.1007/s007020070069
23. Saito T, Lachman HM, Diaz L, Hallikainen T, Kauhanen J, Salonen JT, Ryynänen OP, Karvonen MK, Syvälahti E, Pohjalainen T, Hietala J, Tiihonen J (2002) Analysis of monoamine oxidase A (MAOA) promoter polymorphism in Finnish male alcoholics. Psychiatry Res 109(2):113–119. https://doi.org/10.1016/s0165-1781(02)00013-6
24. Nakamura K, Sekine Y, Takei N, Iwata Y, Suzuki K, Anitha A, Inada T, Harano M, Komiyama T, Yamada M, Iwata N, Iyo M, Sora I, Ozaki N, Ujike H, Mori N (2009) An association study of monoamine oxidase A (MAOA) gene polymorphism in methamphetamine psychosis. Neurosci Lett 455(2):120–123. https://doi.org/10.1016/j.neulet.2009.02.048
25. Parsian A (1999) Sequence analysis of exon 8 of MAO-A gene in alcoholics with antisocial personality and normal controls. Genomics 55(3):290–295. https://doi.org/10.1006/geno.1998.5664
26. Parsian A, Cloninger CR, Sinha R, Zhang ZH (2003) Functional variation in promoter region of monoamine oxidase A and subtypes of alcoholism: haplotype analysis. American journal of medical genetics. Part B, neuropsychiatric genetics: the official publication of the International Society of Psychiatric Genetics. 117B(1):46–50. https://doi.org/10.1002/ajmg.b.10017
27. Chester DS, DeWall CN, Derefinko KJ, Estus S, Peters JR, Lynam DR, Jiang Y (2015) Monoamine oxidase A (MAOA) genotype predicts greater aggression through impulsive reactivity to negative affect. Behav Brain Res 283:97–101. https://doi.org/10.1016/j.bbr.2015.01.034
28. Canan F, Kuloglu M, Guven M, Gecici O (2015) Reliability and validity of the Turkish version of the clinical opiate Withdrawal Scale (COWS). Klinik Psikofarmakoloji Bülteni-Bulletin Clin Psychopharmacol 25(3):267–271
29. Evren C, Durkaya M, Çelik R, Dalbudak E, Çakmak D, Flannary B (2008) Penn Alkol Aşerme Ölçeği (PAAÖ) Türkçe şeklinin yatarak tedavi gören erkek alkol bağımlısı hastalarda geçerliği ve güvenirliği. Bağımlılık Dergisi 9(3):128–134
30. Hisli N (1989) A reliability and validity study of Beck Depression Inventory in a university student sample. J Psychol 7:3–13
31. Ulusoy M, Sahin NH, Erkmen H (1998) Turkish version of the Beck anxiety inventory: psychometric properties. J Cogn Psychother 12(2):163
32. Huang SY, Lin WW, Wan FJ, Chang AJ, Ko HC, Wang TJ, Wu PL, Lu RB (2007) Monoamine oxidase-A polymorphisms might modify the association between the dopamine D2 receptor gene and alcohol dependence. J Psychiatry Neuroscience: JPN 32(3):185–192
33. Kaya H, Kaya ÖB, Kahve AC, Dilbaz N (2020) Evaluation of the relationship between MAOA-uVNTR gene polymorphism and impulsivity, anger, temperament and personality traits in healthy male subjects. Annals Med Res 27(12):3136–3142
34. Koller G, Bondy B, Preuss UW, Bottlender M, Soyka M (2003) No association between a polymorphism in the promoter region of the MAOA gene with antisocial personality traits in alcoholics. Alcohol Alcohol (Oxf Oxfs) 38(1):31–34. https://doi.org/10.1093/alcalc/agg003
35. Laqua C, Zill P, Koller G, Preuss U, Soyka M (2015) Assoziation Des MAOA-uVNTR-Polymorphismus mit antisozialem Verhalten Bei alkoholabhängigen Männern [Association between the MAOA-uVNTR polymorphism and antisocial personality traits in alcoholic men]. Fortschr Neurol Psychiatr 83(3):162–169. https://doi.org/10.1055/s-0034-1399249
36. Cases O, Seif I, Grimsby J, Gaspar P, Chen K, Pournin S, Müller U, Aguet M, Babinet C, Shih JC (1995) Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. Sci (New York N Y) 268(5218):1763–1766. https://doi.org/10.1126/science.7792602
37. Brunner HG, Nelen M, Breakefield XO, Ropers HH, van Oost BA, Science (1993) (New York, N.Y.) 262(5133): 578–580. https://doi.org/10.1126/science.8211186
38. Meyer-Lindenberg A, Buckholtz JW, Kolachana B, Hariri R, Pezawas A, Blasi L, Wabnitz G, Honea A, Verchinski R, Callicott B, Egan JH, Mattay M, Weinberger V DR (2006) Neural mechanisms of genetic risk for impulsivity and violence in humans. Proc Natl Acad Sci USA 103(16):6269–6274. https://doi.org/10.1073/pnas.0511311103
39. Verma D, Chakraborti B, Karmakar A, Bandyopadhyay T, Singh AS, Sinha S, Chatterjee A, Ghosh S, Mohanakumar KP, Mukhopadhyay K, Rajamma U (2014) Sexual dimorphic effect in the genetic association of monoamine oxidase A (MAOA) markers with autism spectrum disorder. Prog Neuro-psychopharmacol Biol Psychiatry 50:11–20. https://doi.org/10.1016/j.pnpbp.2013.11.010
40. Sampaio AS, Hounie AG, Petribú K, Cappi C, Morais I, Vallada H, do Rosário MC, Stewart SE, Fargeness J, Mathews C, Arnold P, Hanna GL, Richter M, Kennedy J, Fontenelle L (2015) Bragança Pereira CA, Pauls DL, Miguel EC COMT and MAO-A polymorphisms and obsessive-compulsive disorder: a family-based association study. PloS one 10(3):e0119592. https://doi.org/10.1371/journal.pone.0119592
41. Karmakar A, Maitra S, Verma D, Chakraborti B, Goswami R, Ghosh P, Sinha S, Mohanakumar KP, Usha R, Mukhopadhyay K (2014) Potential contribution of monoamine oxidase a gene variants in ADHD and behavioral co-morbidities: scenario in eastern Indian probands. Neurochem Res 39(5):843–852. https://doi.org/10.1007/s11064-014-1276-4
42. Karmakar A, Goswami R, Saha T, Maitra S, Roychowdhury A, Panda CK, Sinha S, Ray A, Mohanakumar KP, Rajamma U, Mukhopadhyay K (2017) Pilot study indicate role of preferentially transmitted monoamine oxidase gene variants in behavioral problems of male ADHD probands. BMC Med Genet 18(1):109. https://doi.org/10.1186/s12881-017-0469-5
43. Currie SR, Patten SB, Williams JV, Wang J, Beck CA, El-Guebaly N, Maxwell C (2005) Comorbidity of major depression with substance use disorders. Can J Psychiatry 50(10):660–666. https://doi.org/10.1177/070674370505001013
44. Alsheikh AM, Elemam MO, El-Bahnasawi M (2020) Treatment of Depression with Alcohol and Substance Dependence: a systematic review. Cureus 12(10):e11168. https://doi.org/10.7759/cureus.11168
45. Chen KW, Banducci AN, Guller L, Macatee RJ, Lavelle A, Daughters SB, Lejuez CW (2011) An examination of psychiatric comorbidities as a function of gender and substance type within an inpatient substance use treatment program. Drug Alcohol Depend 118(2–3):92–99. https://doi.org/10.1016/j.drugalcdep.2011.03.003
46. Dierker L, Selya A, Lanza S, Li R, Rose J (2018) Depression and marijuana use disorder symptoms among current marijuana users. Addict Behav 76:161–168. https://doi.org/10.1016/j.addbeh.2017.08.013
47. Suter M, Strik W, Moggi F (2011) Depressive symptoms as a predictor of alcohol relapse after residential treatment programs for alcohol use disorder. J Subst Abuse Treat 41(3):225–232. https://doi.org/10.1016/j.jsat.2011.03.005
48. Jordans MJD, Luitel NP, Garman E, Kohrt BA, Rathod SD, Shrestha P, Komproe IH, Lund C, Patel V (2019) Effectiveness

of psychological treatments for depression and alcohol use disorder delivered by community-based counsellors: two pragmatic randomised controlled trials within primary healthcare in Nepal. Br J Psychiatry: J Mental Sci 215(2):485–493. https://doi.org/10.1192/bjp.2018.300

49. Anand D, Paquette C, Bartuska A, Daughters SB (2019) Substance type moderates the longitudinal association between depression and substance use from pre-treatment through a 1-year follow-up. Drug Alcohol Depend 197:87–94. https://doi.org/10.1016/j.drugalcdep.2019.01.002
50. Volkow ND (2004) The reality of comorbidity: depression and drug abuse. Biol Psychiatry 56(10):714–717. https://doi.org/10.1016/j.biopsych.2004.07.007